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(71) Applicant (for all designated States except US): BRITISH  
TEXTILE TECHNOLOGY GROUP [GB/GB]; Shirley  
Towers, 856 Wilmslow Road, Didsbury, Manchester M20  
8RX (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SAGAR, Brian, Frederick  
[GB/GB]; The Cottage, 42 Moseley Road, Cheadle Hulme,  
Stockport, Cheshire SK8 5HJ (GB). SAGAR, Anthony,  
John, Grant [GB/GB]; 50 Valley Road, Bramhall, Stockport,  
Cheshire SK7 2NL (GB). GRAHAM, Samuel, Gordon  
[GB/GB]; 2 Akesmoor Drive, Mile End Lane, Stockport,  
Cheshire SK2 6BU (GB). WRAGG, Reginald, Trevor  
[GB/GB]; The Wickets, Church Road, Elford, Tamworth,  
Staffordshire B79 9DA (GB).

(74) Agents: McNEIGHT, David, Leslie et al; McNeight &  
Lawrence, Regent House, Heaton Lane, Stockport, Cheshire  
SK4 1BS (GB).

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(54) Title: METHOD OF ENCAPSULATING SUBSTANCES IN BIOCAPSULES

(57) Abstract

There is disclosed a method for encapsulating a substance in a biocapsule comprising passing a solution into the biocapsule, then effecting a change in the biocapsule/solution system such that the substance remains encapsulated while a solvent escapes.

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## METHOD OF ENCAPSULATING SUBSTANCES IN BIOCAPSULES.

This invention relates to methods for encapsulating substances.

It is known to encapsulate various substances in microcapsules which occur naturally or which can otherwise be referred to as biocapsules. US-A-4 696 863, GB-A-2 162 147 and EP-A-0 242 135 disclose bioencapsulation in such organisms as yeast cells (which may be used alive or dead), microfungi, bacteria and algae.

Difficulties are encountered when trying to encapsulate certain substances. GB-A-2 234 901 discloses a method for encapsulating water-insoluble substances which are, however, soluble in liquid ammonia, which is used as the carrier by which the substance is carried by diffusion into the cell.

It is now found that even some water soluble substances cannot effectively be encapsulated using an aqueous solution, because the substances pass out of the capsule as easily as they are passed in, and the present invention provides a generalised method which can be used with such and other difficult-to-encapsulate substances.

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The invention comprises a method for encapsulating a substance in a biocapsule comprising passing a solution into the biocapsule, then effecting a change in the biocapsule/solution system such that the substance remains encapsulated while a solvent escapes.

The change may comprise a physical change, which ~~may involve an increase in solution concentration, at~~ least as a proportion of solution saturation level, within the capsule. It may involve a decrease in solubility of the substance in the solution within the capsule, as by cooling, and the substance may precipitate from a saturated solution within the capsule.

The solvent may however evaporate from the capsule, leaving behind the substance.

The solution may swell the biocapsule sufficiently to pass through the capsule wall to enter the capsule, and the change may then comprise a reversal of the capsule swelling sufficient to trap molecules of the substance but allow solvent molecules to pass out.

The change may comprise a chemical change. A first solution may be passed into the biocapsule, then a second solution passed into the biocapsule which reacts with the first solution to yield the substance in the biocapsule in such manner as to remain encapsulated.

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The first and second solutions may comprise different solutes, or different solvents, or both.

Liquid ammonia, such as has been proposed for water-insoluble substances as noted above, is found now also to be useful for certain water-soluble substances which, however, cannot be encapsulated from aqueous solution for the reason stated above. It is especially suitable for use with some acids and in particular with amino acids.

Amino acids may be in the salt form, preferably the ammonium salt, and may be encapsulated alone or together with other ingredients, for example fish oils. In addition to increasing the nutritional value of such capsules, fish oils also protect the amino acids from leaching out of the capsules. For example, the amino acid histidine may be encapsulated as follows :-

Histidine (L- $\alpha$ -amino-B-imidazolepropionic acid)  
5g, is dissolved in liquid ammonia 150 g. Yeast  
25 g, is added slowly with continuous stirring.  
After 5 minutes the mixture is poured into  
absolute ethanol 600 ml and the resultant  
mixture filtered and washed with absolute  
ethanol und r gentle suction. The filter cake  
is spread out and allowed to dry in air.

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Analysis revealed approximately 12% w/w of histidine encapsulated by the yeast after this process was carried out.

Materials encapsulated in starch or yeast granules, for example, may be used as animal feeds, in particular for ruminant feeds where the capsule coating would protect its contents, ie. amino acids, during passage through the first stomach (rumen), where normally they would be destroyed, until they entered the second stomach (abomasum) where they may be absorbed intact.

In addition, such capsules may be used as feed for fish larvae, and the content of the capsules could be tailored specifically for the particular needs of each stage of the growing larvae.

Many other substances may be encapsulated, for example :

Enzymes could be encapsulated to be put in or on materials needing some degree of digestion, for example, in washing powders;

Perfumes or optical brighteners may be encapsulated in spray starches;

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Biocidal agents, for example insecticides, may be encapsulated and used in a dust form rather than the commonly used pellets, i.e. metaldehyde pellets for killing slugs, to prevent ingestion by other animals such as birds;

Plant fertilisers may be encapsulated, the rate of release being regulated by the type of capsule coating used;

Intumescent flame retardant, i.e. two or more different reactive compounds which produce an expanded flame retardant, may be encapsulated separately;

Oil may be encapsulated in carbohydrate capsules in a proportion such that addition of a fixed amount of water produces a pastry for baking, while other proportions may produce cake mixes and the like.

It will be appreciated that it is not intended to limit the invention to the above examples only, many variations, such as might readily occur to one skilled in the art, being possible, without departing from the scope thereof.

CLAIMS

1. A method for encapsulating a substance in a biocapsule comprising passing a solution into the biocapsule, then effecting a change in the biocapsule/solution system such that the substance remains encapsulated while a solvent escapes.
2. A method according to claim 1, in which the change comprises a physical change.
3. A method according to claim 2, in which the physical change involves an increase in solution concentration within the capsule.
4. A method according to claim 2, in which the physical change involves a decrease in solubility of the substance in the solution within the capsule.
5. A method according to claim 3 or claim 4, in which the substance precipitates from a saturated solution.
6. A method according to claim 2, in which the solvent evaporates from the capsule.

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7. A method according to claim 2, in which the solution swells the bio-capsule and the physical change comprises a reversal of the swelling sufficient to trap molecules of the substance but allow solvent molecules to pass.
8. A method according to any one of claims 1 to 7, in which the substance is dissolved in liquid ammonia.
9. A method according to claim 8, in which the biocapsule/solution system warms to a temperature at which liquid ammonia evaporates.
10. A method according to claim 8 or claim 9, in which the substance is water-soluble but cannot in water solution be retained in the capsule.
11. A method according to claim 10, in which the substance comprises an acid.
12. A method according to claim 11, in which the substance comprises an amino acid.
13. A method according to claim 12, wherein the amino acid is histidine.

14. A method according to claim 1, in which the change comprises a chemical change.

15. A method according to claim 14, in which a first solution is passed into the biocapsule, then a second solution is passed into the biocapsule which reacts with the first solution to yield the substance in the biocapsule in such manner as to remain encapsulated.

16. A method according to claim 15, in which the first and second solutions comprise different solutes.

17. A method according to claim 15 or claim 16, in which the first and second solutions comprise different solvents.

## INTERNATIONAL SEARCH REPORT

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## A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 B01J13/02 A61K9/16 A61K9/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 B01J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 162 147 (DUNLOP) 29 January 1986 cited in the application see claims 1,21,22	1,2,7
X	FR,A,2 179 528 (SEROZYM) 23 November 1973 see claims	1-7
X	US,A,B498208 (SHANK) 13 April 1976 see claims	1,2,7
A	EP,A,0 085 805 (DUNLOP) 17 August 1983 cited in the application	
A	GB,A,2 234 901 (BRITISH TEXTILE TECHNOLOGY GROUP) 20 February 1991 cited in the application see claims	8,9

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Meertens, J

# INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A-2162147	29-01-86	NONE	
FR-A-2179528	23-11-73	NONE	
US-A-B498208	13-04-76	US-A- 4001480 CA-A- 1060364	04-01-77 14-08-79
EP-A-0085805	17-08-83	AU-A- 9072582 JP-A- 58107189	26-05-83 25-06-83
GB-A-2234901	20-02-91	NONE	